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ORIGINAL ARTICLE

Characterization and transcriptional analysis of a subtelomeric satellite DNA family in the ladybird beetle *Henosepilachna argus* (Coleoptera: Coccinellidae)

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Key words. Coleoptera, Coccinellidae, *Henosepilachna argus*, ladybird beetle, satellite DNA, transcription, in situ hybridization

Abstract. Satellite DNAs are the major repetitive DNA components in eukaryotic genomes. Although satellite DNA has long been called "parasite DNA" there is substantial evidence that it could be associated with some functions of chromosome biology. Ladybird beetles (Coccinellidae) are one of the largest and most important groups of beetles. Many ladybirds are of economic interest as biological control agents because they eat some agricultural pests such as aphids and scale insects. However, other species are phytophagous and can damage crops. Despite the ecological importance of the latter group there are no studies on their satellite DNA. A satellite DNA family was isolated and characterized in the ladybird *Henosepilachna argus*. This satellite DNA is organized in tandem repeats of 658 bp and is A + T rich (67.3%). The recorded high sequence conservation of the monomers together with the detection of putative gene conversion processes indicate concerted evolution. Reverse transcription polymerase chain reaction (RT-PCR) revealed that this satellite DNA is transcribed and in situ hybridization its location in the subtelomeric regions of all chromosomes except the long arm of the X chromosome. The presence of this satellite DNA in other species of the genus *Henosepilachna* and *Epilachna* was also tested using PCR. The results indicate that this satellite DNA sequence is so far specific to *H. argus*.

INTRODUCTION

Transposable elements and satellite DNAs are the major repetitive DNA components in eukaryotic genomes. Satellite DNAs are present in long tandem arrays of similar motifs (monomers or repeat units) in a head-to-tail fashion and in some species make up more than half of the total content of the genomic DNA (Plohl et al., 2012). Satellite DNA is located mainly in heterochromatic chromosomal regions, usually around centromeres, but also can be located in the subtelomeric regions as well as the interstitial regions of chromosomes (Li & Lin, 2012). Satellite DNA has long been called "junk" or "parasite DNA", although its functional role is still controversial (Palazzo & Gregory, 2014). Numerous pieces of evidence support the contention that satellite DNA could be involved in certain functions such as centromere structure, kinetochore formation, and chromosome pairing and segregation. The evolution of repetitive DNA has also been related to reproductive isolation and therefore with the appearance of new species (Ferree & Prasad, 2012). Its importance in genome integrity and in karyotypic evolution has also been highlighted (reviewed in Biscotti et al., 2015a, b). Transcription and the possible role of 1,688 satellite DNA transcripts from *Drosophila melanogaster* has been extensively studied (Usakin et al., 2007; Menon et al., 2014; Rošić et al., 2014; among others). Recently Ferree (2017) has suggested, on the basis of previous studies on the role of satellite DNA transcripts and especially those related to the 1,688 satellite DNA transcripts identified in fruit fly, that "satellite DNA and corresponding small non-coding RNA, helps the dosage compensation machinery preferentially to find X sequences". Consequently, in accordance with this author, this satellite DNA "directs male-specific gene expression".

The order Coleoptera is the largest of all insect orders, comprising almost 40% of described species of insects. The estimated number of beetle species is between 300,000 and 450,000 belonging to 211 families (Nielsen & Mound, 1999; Bouchard et al., 2011). However, satellite DNA has been analyzed in only a little more than 50 species of five families, most of them from the family Tenebrionidae (reviewed by Palomeque & Lorite, 2008). Notably, studies on the tenebrionid red flour beetle, *Tribolium castaneum* (Feliciello et al., 2015; Pavlek et al., 2015), indicate that satellite DNA modulates gene expression after heat stress

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(Feliciello et al., 2015). Despite the above, the information on satellite DNA in this insect order is very limited. Ladybirds (Coccinellidae) constitute one of the largest and most important beetle groups, having almost 6000 species with a worldwide distribution (Ślipiński & Tomaszewska, 2010). Many ladybirds are of economic interest as biological control agents because they are predators of some agricultural pests such as aphids and scale insects. On the other hand, other species are phytophagous and can damage crops. Despite the ecological importance of the latter group, there are no studies on the satellite DNA in species of Coccinellidae. The objective of this study is to begin the analysis of satellite DNA in this insect group. In this connection, a family of satellite DNA was isolated and analyzed in the species *Henosepilachna argus* Geoffroy, 1762. This species belongs to the tribe Epilachnini. All the species in the tribe Epilachnini are herbivorous and some are pests of important crops such as eggplant, squash and beans (Schaefer, 1983).

MATERIAL AND METHODS

Material

This study was carried out with wild *Henosepilachna argus* collected on *Ecballium elaterium* plants, in the province of Jaén (Spain). For the comparative analysis, other Epilachnini species were used: *Henosepilachna vigintioctomaculata* Motschulsky, 1857 (Hokkaido, Japan), *Henosepilachna septima* Dieke, 1947 (Islamabad, Pakistan), *Epilachna paenulata* Germar, 1824 (Montevideo, Uruguay) and *Epilachna admirabilis* Crotch, 1874 (Hadano, Japan).

Extraction of genomic DNA and isolation of repetitive DNA

Genomic DNA was isolated using a commercial kit (Machery-Nagel GmbH & Co., Düren, Germany), following the instructions provided by the manufacturer. Each DNA extraction was performed using 2 to 4 adults.

Genomic DNA was digested overnight with a battery of restriction endonucleases using 4 U/µg DNA. Digested DNA was analyzed by means of electrophoresis using 2% agarose gels. Fragments of about 650 bp produced by digestion of genomic DNA with *Msp*I were eluted from the agarose gel and inserted into the pGEM-T Easy Vector (Promega, Madison, WI, USA). Compatible and cohesive ends were generated by incubating the isolated band with *Taq* polymerase (Sánchez et al., 1996). A sample of the ligation reaction was used for the transformation of competent *Escherichia coli* DH5α (Zymo Research, Orange, CA, USA). Recombinants were selected using LB/ampicillin/IPTG/X-Gal plates. The recombinant plasmids were purified using the EZNA Plasmid Midi Kit (Omega, Norcross, GA, USA). A sample of the 650-bp fragments was labelled with DIG-11-dUTP using the DIG DNA labelling Kit (Roche, Basel, Switzerland) and used for plasmid screening. Selected recombinant plasmids were directly sequenced on both strands.

A second set of repeated sequences was obtained using PCR. The primers HargM-PCR-1 (5′-ATATTTGCCTGAATTATGCG) and HargM-PCR-2 (5′-TGAAATGCACGCTCACAG) were designed from conserved regions of the sequences obtained from the cloned 650 bp band. PCRs were performed in 25 µl of reaction mixture containing 50 ng of genomic DNA, 10 pmol of each primer and 0.75 U of *Taq* polymerase. The PCR program used was 1 min at 92°C and 35 cycles: 30 s at 92°C, 30 s at 50°C, 1 min at 72°C, with a final extension of 5 min at 72°C. The PCR-obtained bands were inserted into the pGEM-T Easy vector (Promega). Plasmids with inserted sequences were sequenced on both strands.

Satellite DNA sequence analysis

Multiple-sequence alignments were performed using CLUSTALW (Thompson et al., 1994). The sequence data were analyzed and compared with the GenBank/NCBI DNA databases using the BLAST network service and the EMBL database (Altschul et al., 1997). The DnaSP5 (Librado & Rozas, 2009) program was used to identify putative gene conversion events. For this analysis, it is necessary to define the two sets of sequences that are compared. One set must contain at least three sequences and the other at least five sequences. Several sets of sequences were defined and tested for gene conversion. The evolutionary divergence between sequences was estimated using the *p*-distance model and the program MEGA version 6 (Tamura et al., 2013).

Southern hybridization

First, 4 µg of genomic DNA were digested overnight with restriction endonucleases (4 U/µg DNA) and separated on 2% agarose gels. After electrophoresis, the digested DNA was transferred onto nylon membranes using standard procedures. Southern hybridization was performed at 60°C using 20 ng of labelled probe/ml. The insert of the clone HargM-12 was amplified using universal primers T7 and SP6. The PCR product was labelled with DIG-11-dUTP using the DIG DNA labelling Kit (Roche) and used as a probe. Hybridization was detected using the DIG-detection kit (Roche) and a final wash in 2 × SSC at 60°C.

Fluorescence in situ hybridization

Chromosome slides were obtained from adult male gonads as described in Lorite et al. (1996). Fluorescence in situ hybridization (FISH) using the insert of the HargM-46 clone was performed as described in Palomeque et al. (2005). The probe was labelled with biotin-16-dUTP using the Nick Translation Kit (Roche), according to the manufacturer's instructions (final concentration of 2 ng/ml in 50% formamide). The fluorescence immunological detection was made using the avidin-FITC/anti-avidin-biotin system with one amplification round. Slides were mounted in Vectashield – DAPI (Vector Laboratories, Burlingame, CA, USA). DAPI in the antifade solution was used to counterstain the chromosomes. Images were made and analyzed using a BX51 Olympus® fluorescence microscope (Olympus, Hamburg, Germany) equipped with a CCD camera (Olympus® DP70) and processed using Adobe® Photoshop® software.

RT-PCR

For RT-PCR, total RNA was extracted from two individuals of *H. argus* using the Direct-sol RNA MiniPrep (Zymo Research) following the manufacturer's protocol. RNA was eluted in 35 µl of DNase/RNase-Free water and 14 µl from each sample were used to generate cDNA using a Maxima First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). As a control, a sample of RNA was treated with 2 U of DNAse-free RNAase (Invitrogen, Carlsbad, CA, USA) at 37°C for 20 min.

PCRs were performed using 5 µl of each of the above two samples. For PCR amplification the primers HargM-PCR-1 and HargM-PCR-2 were used. PCRs were performed in 25 µl using 10 pmol of each primer and 1 U of *Taq* polymerase (Bioline, London, UK). The PCR program used was 2 min at 92°C, and 40 cycles of 30 s at 92°C, 30 s at 56°C, 60 s at 72°C for 35 cycles, with a final extension at 72°C for 5 min. The PCR products were inserted into the pGEM-T Easy vector (Promega) and sequenced as indicated above.

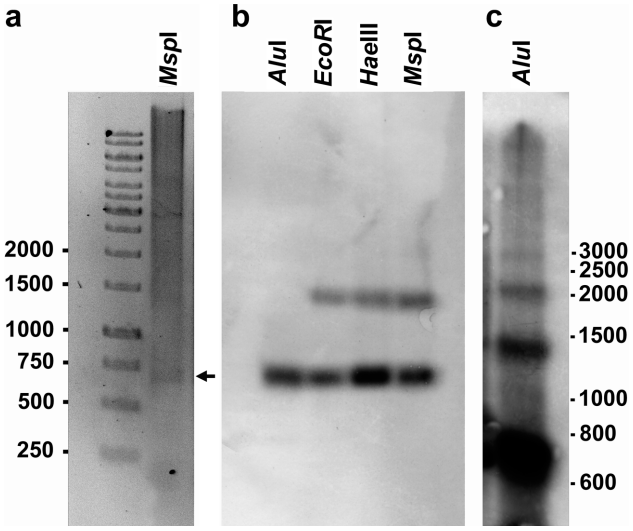

Fig. 1. (a) Electrophoretic separation on 2% agarose gels of *H. argus* genomic DNA after digestion with *Msp*I, showing a band of repetitive DNA of about 650 bp (arrow). The numbers on the left indicate the size in bp of the DNA fragments in the size marker lane. (b) Southern-blot hybridization using genomic DNA digested with several restriction endonucleases using the HargM-12 clone as a probe. The 650-bp band is visible in all lanes as well as a second band, probably a dimer, in the last three lanes. (c) Southern-blot hybridization using genomic DNA partially digested with *Alu*I, showing the presence of the typical ladder for satellite DNAs.

RESULTS AND DISCUSSION

Digestion of genomic DNA with *Msp*I revealed a band of repetitive DNA of about 650 bp (Fig. 1a). This band was eluted and cloned into the pGEM-T vector. Recombinant plasmids were named HargM (*Henosepilachna argus Msp*I). After screening, seven plasmids were selected and sequenced; HargM-2, -12, -27, -46, -80, -90 and -94 (EMBL accession numbers LT896703-709). The alignment of the sequences of the inserts of these plasmids is shown in Fig. 2. These sequences are not similar to other repeat DNAs in the sequence data banks.

To determine whether the isolated repetitive DNA was dispersed or was a tandemly organized satellite DNA, we used two analyses: PCR amplification and Southern hybridization. For PCR, two primers were designed based on the sequence of the HargM repeats. If the c. 650 bp sequences were in tandem, these primers could amplify the DNA located between two of these fragments. As expected for a tandem repeat, PCR generates bands of about 150 and 800 bp (Fig. 3a). The 800-bp band was cloned and five clones were sequenced (HargM-PCR-281, -282, -286, -287 and -295) (EMBL accession nos. LT896710-714). The sequences of the PCR-generated fragments are also depicted in Fig. 2.

Southern hybridization was carried out with genomic DNA digested using four restriction endonucleases (*Alu*I, *Eco*RI, *Hae*III and *Msp*I). These four enzymes were selected since their targets were present in the cloned sequences. Southern hybridization of a satellite DNA generates the appearance of a ladder of bands, generated by mutations in the restriction target, giving rise to the appearance of dimers, trimers, tetramers, etc. Southern hybridization using the repeat HargM-2 as a probe generated the appearance of a band of about 650 bp for the four enzymes used (Fig. 1b). In DNA digested using *Eco*RI, *Hae*III and *Msp*I a second band, probably a dimer, is also visible. The absence of larger bands (trimers, tetramers, etc.) could be a consequence of the high level of conservation of the sequence of this satellite DNA family, which would generate a very low number of fragments to be detected by Southern-blot, as is reported for highly conserved satellite DNA families in the Colorado potato beetle, *Leptinotarsa decemlineata* (Lorite et al., 2013). For a more thorough analysis of the hybridization profile, we partially digested *H. argus* genomic DNA using *Alu*I. In this case a ladder pattern resulted, in which tetramers are clearly visible (Fig. 1c). PCR and Southern-blot results support the idea that this repetitive DNA is a satellite DNA with a tandem repeat of 650 bp.

The alignment of the sequences resulting from digestion with *Msp*I and the complete monomers obtained using PCR amplification is shown in Fig. 2. The sequence of this satellite DNA family is highly conserved and there are no differences between the two groups of sequences (Table 1). The divergence among sequences found by digestion with *Msp*I ranged between 0.6 and 4.7% and among sequences found by PCR between 0.9 and 4.1%. When the sequences of the two groups were compared, similar divergences were recorded of between 0.5 to 4.7%. Thus, similarity among the different monomers ranged between 95.3 and 99.5% (Table 1). This agrees with the results of the Southern-blot hybridization. In highly conserved sequences the numbers of nucleotide changes among monomers was low, so most of the monomers were targets for *Msp*I, and as a consequence the Southern hybridization revealed only a hybridization band. The alignment of the HargM repeats showed that the consensus sequence of this satellite DNA was 658 bp in length and AT-rich (67.3%). Most of the changes recorded are due to point changes, such as indels or substitutions of a single nucleotide. Most of the changes recorded were point mutations present in only one of the sequences. However, the variation was due mainly to mutations shared by several monomers. The mutations shared between several sequences are considered to be generated by a single mutation that subsequently gradually extended to other monomers (Strachan et al., 1985). This evolutionary pattern is the consequence of several processes (gene amplification or deletion, unequal crossing over, slippage replication, gene conversion, rolling circle amplification or transposition) that cause the different repeated units of the satellite DNA family to evolve in concert, i.e. so-called "concerted evolution" (Dover, 2002). In our study, putative gene conversion tracts were detected using the DnaSP5 program. They were found in HargM-2 (tract located between sites 18-414), HargM-80 (located between sites 18-414 and 118-493), HargM-PCR-281 (located between sites 60-164) and HargM-70 (located between sites 433–450), among others. Several authors have recently highlighted the importance of gene conversion processes in satellite DNA evolution (Khost et al., 2017; Lorite et al., 2017),

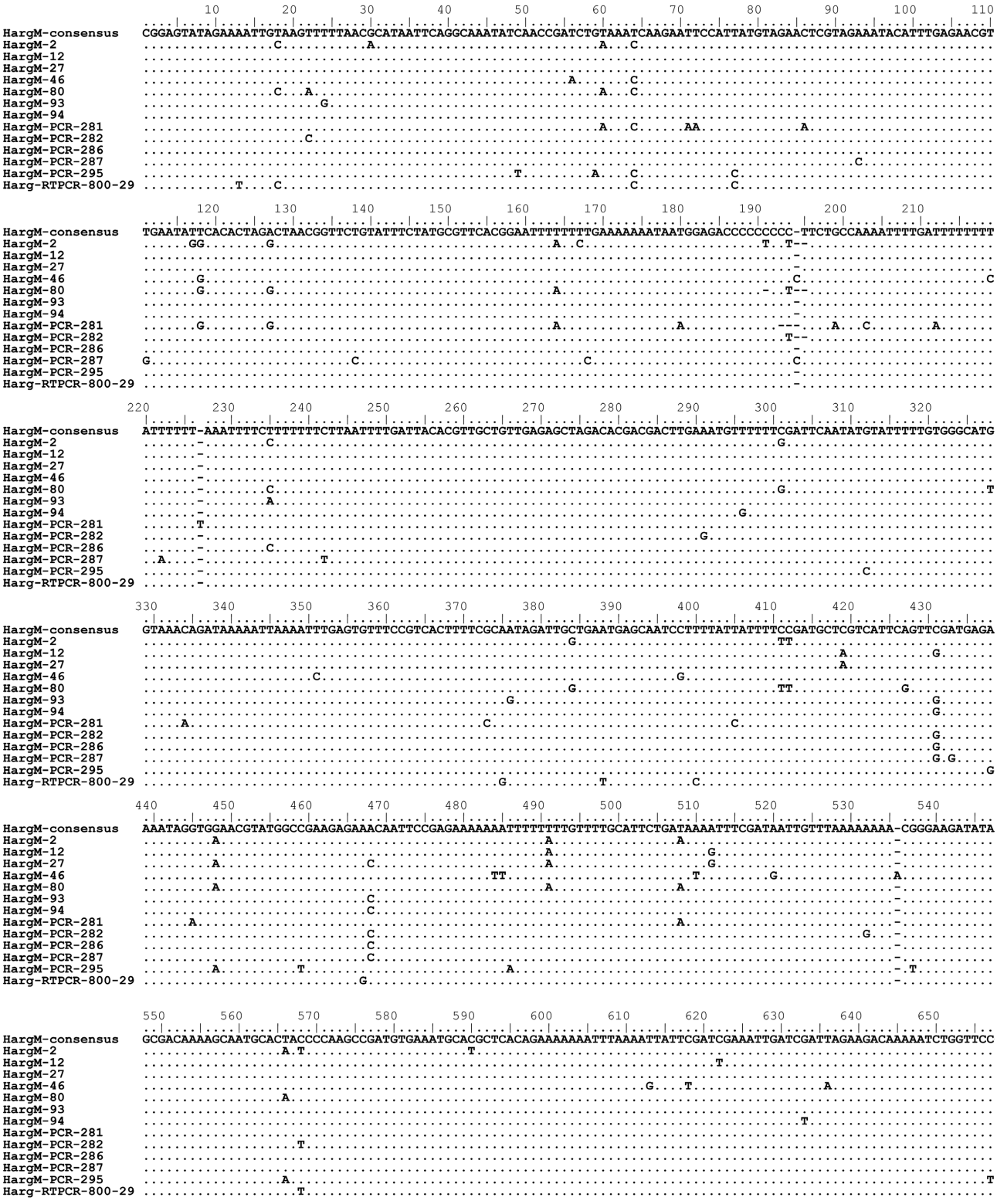

Fig. 2. Alignment of HargM sequences and the consensus sequence derived from them. The first seven sequences were obtained from the band generated by digestion of *H. argus* genomic DNA with *Msp*I (HargM-2 to -94). The following sequences (HargM-PCR) were revealed by PCR. The last sequence (Harg-RT-PCR-800-29) resulted from cloning the band generated by RT-PCR. For PCR and RT-PCR clones only complete monomers were included in the alignment.

supporting the theoretical models that suggest that the unequal crossing-over and gene conversion processes are the most important and widespread mechanisms involved in the evolutionary dynamics of satellite DNAs (Talbert & Henikoff, 2010). The high conservation of HargM monomers together with the detection of putative gene conversion processes indicates that concerted evolution has been important in the evolution of the satellite DNA in *H. argus*.

Recently numerous studies have reported the existence of RNAs transcribed from satellite DNAs of different species

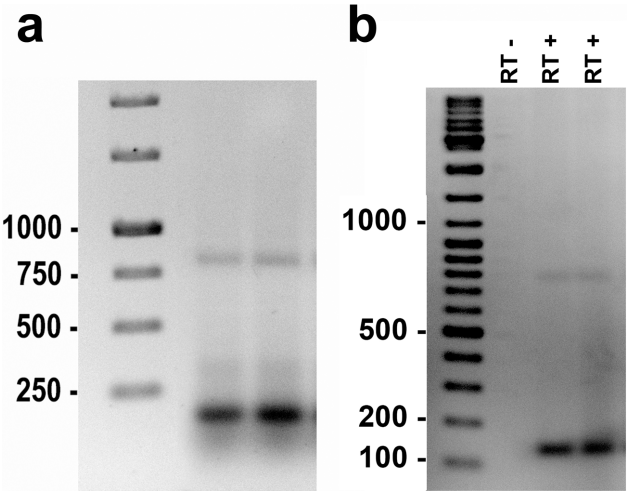

Fig. 3. (a) PCR of genomic DNA of *H. argus* using primers for the HargM satellite DNA family. (b) Analysis of satellite DNA transcription. Lanes RT+ are the RT-PCR products obtained using total RNA extracted from adult insects. Lane RT– is a control with RNA treated with RNase prior to the PCR. The numbers on the left indicate the size of DNA fragments in bp of the size marker lanes.

of vertebrates, invertebrates and plants. RT-PCR was carried out to determinate whether the HargM satellite DNA family is transcribed. If this satellite DNA is transcribed, at least two bands of 150 and 800 bp would be expected. Fig. 3b shows that both expected bands are present. The 800-bp band was cloned and sequenced in order to confirm that it was the expected satellite DNA (Fig. 2) (EMBL accession no. LT896715). The role of satellite DNA transcription is one of the most debated issues related to satellite DNA (reviewed in Biscotti et al., 2015a), as indicated in the Introduction. Furthermore, we highlight recent studies on insects in which differential transcription was detected at different stages of development, although its function is currently unknown (Lima et al., 2017, among others). Despite the foregoing, and as indicated above, the transcripts of the 1,688 satellite DNA from *Drosophila melanogaster* seem to have key roles, making their study highly relevant for structural, functional and evolutionary genomics (Kuhn, 2015).

Henosepilachna argus has a chromosome number of 2n = 18 with an Xy parachute sex chromosome system. All chromosomes have a pericentromerically located prominent heterochromatic block. DAPI staining indicates that this heterochromatin is A + T rich (Mora et al., 2015). Fluorescent in situ hybridization was carried out to determine the chromosomal location of HargM satellite DNA. After the hybridization, the signals appeared in the subtelomeric regions of all autosomes (Fig. 4). On the small "y" chromosome the signals are present on both arms. However, one chromosome lacks the signal on the long arm in all the metaphases analyzed. This chromosome could be the "X" chromosome because there is only one chromosome that lacks a signal. Hybridization with HargM probes indicates that this satellite DNA is not present in the A + T rich pericentromeric heterochromatin, revealed by C-banding and intensely dyed with DAPI. This result indicates that another satellite DNA family could exist, as it is a major component of those pericentromeric regions. Most of the satellite DNA families isolated so far in Coleoptera are located in the heterochromatic pericentromeric regions on all chromosomes, including the sex chromosomes (reviewed by Palomeque & Lorite, 2008; Biscotti et al., 2015b). However, in other species of Coleoptera the satellite DNA is located on all autosomes but absent on one or both sex chromosomes, or located only on several pairs of autosomes (Juan et al., 1993; Pons et al., 2002; Lorite et al., 2002, 2013). In the tenebrionid *Misolampus goudoti* two satellite DNA families have been isolated; one is located in the pericentromeric regions of all chromosomes except the Y chromosome, and the other in the pericentromeric and subtelomeric regions of all chromosomes (Juan et al., 1993). The HargM satellite isolated for *H. argus* is the first in the literature to be located exclusively in subtelomeric regions.

The tribe Epilachnini is poorly represented in Europe, with one species of the genus *Subcoccinella* and three species of *Henosepilachna*, one being *H. argus* (Fauna Europaea, https://fauna-eu.org/). However, Epilachnini, with over 1000 species, contains nearly 20% of the species of Coccinellidae (Tomaszewska & Szawaryn, 2016). Within the tribe Epilachnini the genera *Epilachna* and *Henosepilachna* are the genera richest in species. The distinctiveness of the two genera is one of the most controversial subjects in Epilachnini (Pang et al., 2012; Tomaszewska & Szawaryn, 2016). Recent phylogenetic studies including a wide representation of Epilachnini species have shown that both *Epilachna* and *Henosepilachna* are polyphyletic (Katoh et al., 2014; Szawaryn et al., 2015). Katoh et al. (2014)

Table 1. Estimates of the evolutionary divergence between sequences using the *p*-distance model.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
(1) HargM-2													
(2) HargM-12	0.036												
(3) HargM-27	0.033	0.006											
(4) HargM-46	0.046	0.028	0.028										
(5) HargM-80	0.012	0.033	0.030	0.043									
(6) HargM-90	0.038	0.012	0.012	0.028	0.034								
(7) HargM-94	0.038	0.011	0.011	0.026	0.034	0.008							
(8) HargM-RT-PCR-800-29	0.036	0.022	0.022	0.031	0.036	0.022	0.020						
(9) HargM-PCR-281	0.039	0.034	0.034	0.041	0.036	0.034	0.033	0.038					
(10) HargM-PCR-282	0.038	0.014	0.014	0.030	0.036	0.011	0.009	0.020	0.036				
(11) HargM-PCR-286	0.033	0.009	0.009	0.025	0.030	0.005	0.005	0.019	0.031	0.008			
(12) HargM-PCR-287	0.046	0.019	0.019	0.034	0.043	0.015	0.014	0.028	0.041	0.017	0.012		
(13) HargM-PCR-295	0.041	0.027	0.023	0.036	0.038	0.027	0.025	0.027	0.043	0.028	0.023	0.033	

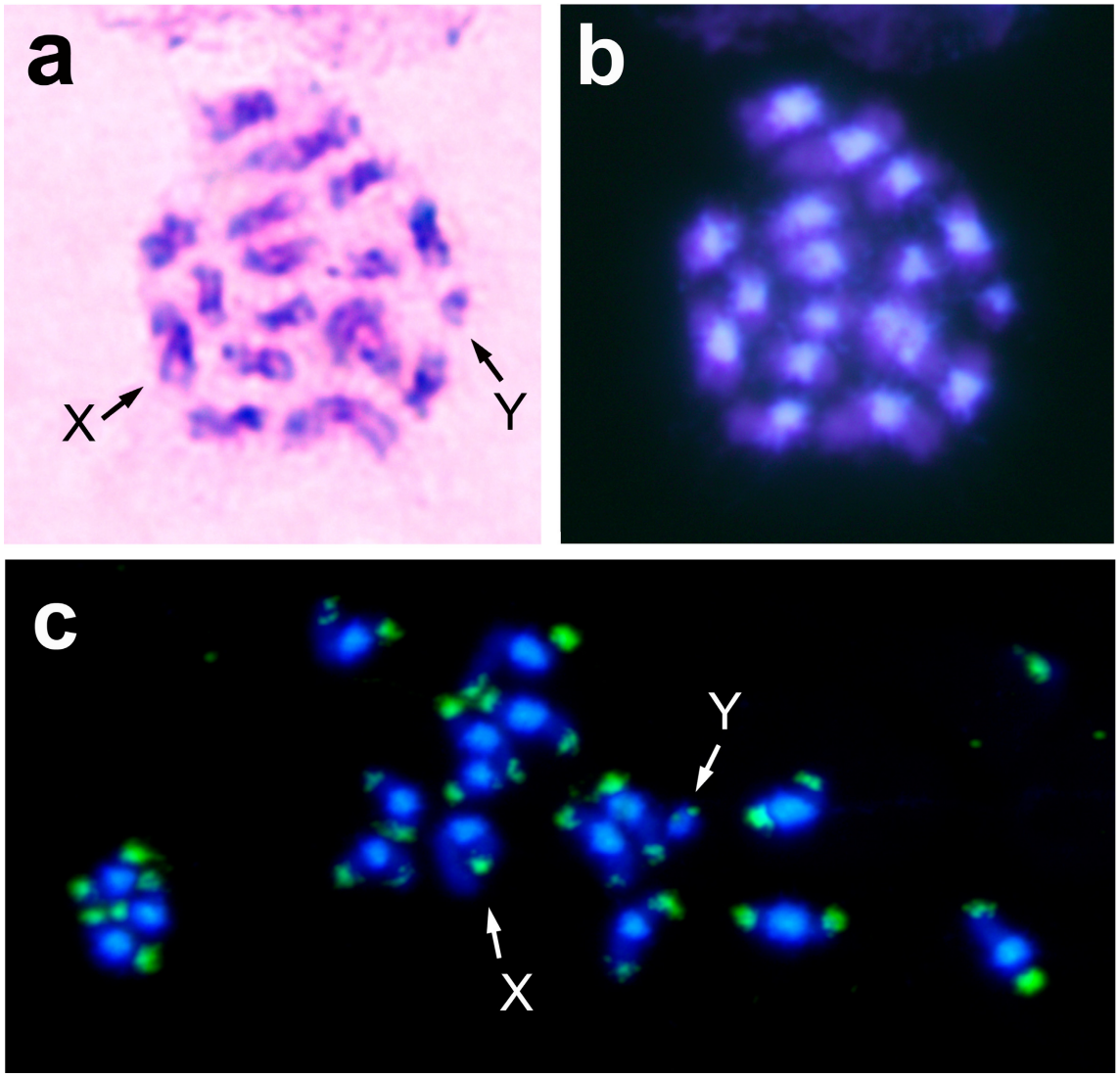

Fig. 4. Mitotic metaphase stained with Giemsa (a) and subsequently with DAPI (b) showing the existence of intensely stained regions coincident with the heterochromatic pericentromeric regions on all chromosomes. (c) FISH using HargM satellite DNA as a probe. Positive hybridization signals (green) are visible in the subtelomeric regions on all chromosomes, with the exception of the long arm of the X chromosome.

have reported that three groups of species of Epilachnini cluster together in well-supported clades: Asian *Epilachna*, American *Epilachna* and Asian-Australian *Henosepilachna*. Using PCR, we have tested for the presence of the HargM satellite DNA using the primers HargM-PCR-1 and HargM-PCR-2 in species included in these clades: *E. admirabilis* (Japan), *E. paenulata* (Uruguay), *H. vigintiotomaculata* (Japan) and *H. septima* (Pakistan). PCR failed to amplify this satellite DNA in these four species indicating that this satellite DNA family could be specific to *H. argus*. Further studies are needed to determine the presence of this satellite DNA family in other species, especially in species close to *H. argus*. Unfortunately, no molecular phylogeny performed so far has included this species, so it is not possible to identify the closest species.

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REFERENCES

Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W. & Lipman D.J. 1997: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. — *Nucl. Acids Res.* **25**: 3389–3402.

Biscotti M.A., Canapa A., Forconi M., Olmo E. & Barucca M. 2015a: Transcription of tandemly repetitive DNA: functional roles. — *Chromos. Res.* **23**: 463–477.

Biscotti M.A., Olmo E. & Heslop-Harrison J.S. 2015b: Repetitive DNA in eukaryotic genomes. — *Chromos. Res.* **23**: 415–420.

Bouchard B., Bousquet Y., Davies A.E., Alonso-Zarazaga M.A., Lawrence J.F., Lyal C.H.C., Newton A.F., Reid C.A.M., Schmitt M., Ślipiński S.A. & Smith A.B.T. 2011: Family-group names in Coleoptera. — *Zookeys* **88**: 1–972.

Dover G. 2002: Molecular drive. — *Trends Genet.* **18**: 587–589.

Feliciello I., Akrap I. & Ugarković D. 2015: Satellite DNA modulates gene expression in the beetle *Tribolium castaneum* after heat stress. — *PLoS Genet.* **11**: e1005466, 18 pp.

Ferree P.M. 2017: Sex differences: satellite DNA directs male-specific gene expression. — *Curr Biol.* **27**: R378–R380.

Ferree P.M. & Prasad S. 2012: How can satellite DNA divergence cause reproductive isolation? Let us count the chromosomal ways. — *Genet. Res. Int*. **2012**: 430136, 11 pp.

Juan C., Pons J. & Petitpierre E. 1993: Localization of tandemly repeated DNA sequences in beetle chromosomes by fluorescent in situ hybridization. — *Chromos. Res.* **1**: 167–174.

Katoh T., Koji S., Ishida T.A., Matsubayashi K.W., Kahono S., Kobayashi N., Furukawa K., Viet B.T., Vasconcellos-Neto J., Lange C.N., Goergen G., Nakano S., Li N.N., Yu G.Y. & Katakura H. 2014: Phylogeny of *Epilachna*, *Henosepilachna*, and some minor genera of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Coccinellinae: Epilachnini), with an analysis of ancestral biogeography and host-plant utilization. — *Zool. Sci.* **31**: 820–830.

Khost D.E., Eickbush D.G. & Larracuente A.M. 2017: Single-molecule sequencing resolves the detailed structure of complex satellite DNA loci in *Drosophila melanogaster*. — *Genome Res.* **27**: 709–721.

Kuhn G.C.S. 2015: Satellite DNA transcripts have diverse biological roles in *Drosophila*. — *Heredity* **115**: 1–2.

Li Y.C. & Lin C.C. 2012: Cervid satellite DNA and karyotypic evolution of Indian muntjac. — *Genes Genom.* **34**: 7–11.

Librado P. & Rozas J. 2009: DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. — *Bioinformatics* **25**: 1451–1452.

Lima L.G. de, Svartman M. & Kuhn G.C.S. 2017: Dissecting the satellite DNA landscape in three cactophilic *Drosophila* sequenced genomes. — *G3-Genes Genom. Genet.* **7**: 2831–2843.

Lorite P., Chica E. & Palomeque T. 1996: Cytogenetic studies of ant *Linepithema humile* Shattuck (= *Iridomyrmex humilis* Mayr) in European populations. — *Caryologia* **49**: 199–205.

Lorite P., Carrillo J.A., Garnería I., Petitpierre E. & Palomeque T. 2002: Satellite DNA in the elm leaf beetle, *Xanthogaleruca luteola* (Coleoptera, Chrysomelidae): characterization, interpopulation analysis, and chromosome location. — *Cytogenet. Genome Res.* **98**: 302–307.

Lorite P., Torres M.I. & Palomeque T. 2013: Characterization of two unrelated satellite DNA families in the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). — *Bull. Entomol. Res.* **103**: 538–546.

Lorite P., Muñoz-López M., Carrillo J.A., Sanllorente O., Vela J., Mora P., Tinaut A., Torres M.I. & Palomeque T. 2017: Concerted evolution, a slow process for ant satellite DNA: study of the satellite DNA in the *Aphaenogaster* genus (Hymenoptera, Formicidae). — *Org. Divers. Evol.* **17**: 595–606.

Menon D.U., Coarfa C., Xiao W., Gunaratne P.H. & Meller V.H. 2014: siRNAs from an X-linked satellite repeat promote X-chromosome recognition in *Drosophila melanogaster*. — *Proc. Natn. Acad. Sci. U.S.A.* **111**: 16460–16465.

Mora P., Vela J., Sanllorente O., Palomeque T. & Lorite P. 2015: Molecular cytogenetic studies in the ladybird beetle *Henosepilachna argus* Geoffroy, 1762 (Coleoptera, Coccinellidae, Epilachninae). — *Comp. Cytogenet.* **9**: 423–434.

Nielsen E.S. & Mound L.A. 1999: Global diversity of insects: the problems of estimating numbers. In Raven P.H. & Williams T. (eds): *Nature and Human Society: The Quest for a Sustainable World*. National Academic Press, Washington DC, pp. 213–222.

Palazzo A.F. & Gregory T.R. 2014: The case for junk DNA. — *PLoS Genet.* **10**: e1004351, 8 pp.

Palomeque T., Muñoz-López M., Carrillo J.A. & Lorite P. 2005: Characterization and evolutionary dynamics of a complex family of satellite DNA in the leaf beetle *Chrysolina carnifex* (Coleoptera, Chrysomelidae). — *Chromos. Res.* **13**: 795–807.

Palomeque T. & Lorite P. 2008: Satellite DNA in insects: a review. — *Heredity* **100**: 564–573.

Pang H., Ślipiński A., Wu Y. & Zuo Y. 2012: Contribution to the knowledge of Chinese *Epilachna* Chevrolat with descriptions of new species (Coleoptera: Coccinellidae: Epilachnini). — *Zootaxa* **3420**: 1–37.

Pavlek M., Gelfand Y., Plohl M. & Meštrović N. 2015: Genome-wide analysis of tandem repeats in *Tribolium castaneum* genome reveals abundant and highly dynamic tandem repeat families with satellite DNA features in euchromatic chromosomal arms. — *DNA Res.* **22**: 387–401.

Plohl M., Meštrović N. & Mravinac B. 2012: Satellite DNA evolution. — *Genome Dyn.* **7**: 126–152.

Pons J., Juan C. & Petitpierre E. 2002: Higher-order organization and compartmentalization of satellite DNA PIM357 in species of the coleopteran genus *Pimelia*. — *Chromos. Res.* **10**: 597–606.

Rošić S., Kohler F. & Erhardt S. 2014: Repetitive centromeric satellite RNA is essential for kinetochore formation and cell division. — *J. Cell Biol.* **207**: 335–349.

Sánchez A., Bullejos M., Burgos M., Jiménez R. & Díaz de la Guardia R. 1996: An alternative to blunt-end ligation for cloning DNA fragments with incompatible ends. — *Trends Genet.* **12**: 44.

Schaefer P.W. 1983: Natural enemies and host plants of species in the Epilachninae (Coleoptera: Coccinellidae) – a world list. — *Delaware Agri. Exp. Stat. Bull.* **445**: 1–42.

Ślipiński S.A. & Tomaszewska W. 2010: Coccinellidae Latreille, 1802. In Leschen R.A.B., Beutel R.G. & Lawrence J.F. (eds): *Handbook of Zoology. Vol. 2. Coleoptera.* Walter de Gruyter, Berlin/New York, pp. 454–472.

Strachan T., Webb D. & Dover G.A. 1985: Transition stages of molecular drive in multiple-copy DNA families in *Drosophila*. — *EMBO J.* **4**: 1701–1708.

Szawaryn K., Bocák L., Ślipiński A., Escalona H.E. & Tomaszewska W. 2015: Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. — *Syst. Entomol.* **40**: 547–569.

Talbert P.B. & Henikoff S. 2010: Centromeres convert but don't cross. — *PLoS Biol.* **8**: e1000326, 5 pp.

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013: MEGA 6: Molecular evolutionary genetics analysis version 6.0. — *Mol. Biol. Evol.* **30**: 2725–2729.

Tomaszewska W. & Szawaryn K. 2016: Epilachnini (Coleoptera: Coccinellidae) – A revision of the world genera. — *J. Insect Sci.* **169**(1): 101, 91 pp.

Thompson J.D., Higgings D. & Gibson T.J. 1994: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. — *Nucl. Acids Res.* **22**: 4673–4680.

Usakin L., Abad J., Vagin V.V., De Pablos B., Villasante A. & Gvozdev V.A. 2007: Transcription of the 1.688 satellite DNA family is under the control of RNA interference machinery in *Drosophila melanogaster* ovaries. — *Genetics* **176**: 1343–1349.

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